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EVALUATION OF THE EFFECTS OF UTILIZING CARBON DIOXIDE AS A PULP--ETC(U)
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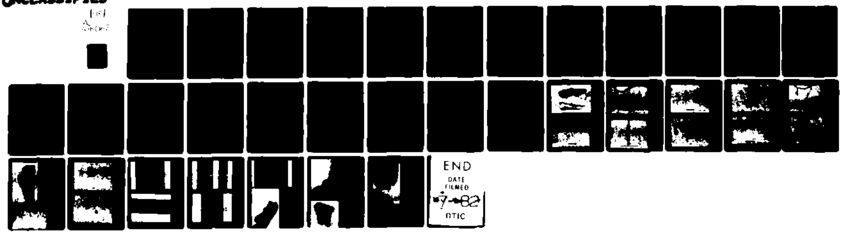

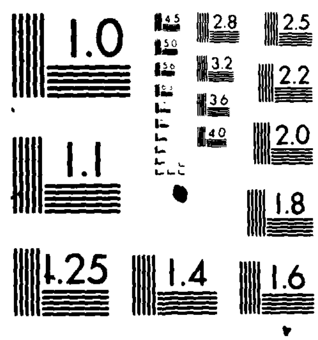

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EVALUATION OF THE EFFECTS OF UTILIZING CARBON DIOXIDE AS A PULPAL TEST. PART I: *IN VITRO* EFFECT ON HUMAN ENAMEL SURFACE

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ABSTRACT

An effective method of clinical pulp testing, carbon dioxide snow was evaluated *in vitro* to determine the effect of the rapid thermal change on the enamel surface. The buccal surfaces of fifteen teeth were subjected to a total of two minutes each of the cold test. No new cracks or fissures were seen after cold application when the surfaces were examined by a profile analyzer, or replicas of the surface by a scanning electron microscope. In only one of nine pre-existing surface fissures could any possible change be shown, and this occurred only after the full two minutes of testing. In respect to use on enamel, carbon dioxide appears to be a safe test.

INTRODUCTION

Thermal testing has proven one of the most predictable tests in endodontics.[1-3] In the United States, for cold thermal testing, dentist have usually used ice carpules[1,4,5] or a skin refrigerant spray.[1,6,7] As previously discussed,[8] melting ice is messy and not very cold. Skin refrigerant is colder and less messy, but it is less efficient in testing multiple teeth.

As early as 1936, dry ice was used for pulp testing.[9] An apparatus was later proposed by Obwegeser and Steinhauser[10] to allow the carbon dioxide (CO_2) to be used in a pencil form. In both their study and a later study,[11] comparing CO_2 snow testing to electrical pulp testing, the accuracy was greater with the CO_2 snow.

Since dry ice is very cold, (-78C, -119F), a question has arisen on the possibility of its causing damage to dental hard structures. Lutz and others,[12] using a dye and fluorescent UV-photography, concluded that a single CO_2 application could accentuate existing enamel cracks. Bachman and Lutz,[13] using the fluorescent technique, compared the effects of the CO_2 snow to a skin refrigerant spray. They found that both reopened or created a few, new fissures. The rate of occurrence was the same for both techniques. They did feel that the CO_2 snow affected the length, width, and depth of the fissures already present more than the skin refrigerant. The changes were small and the clinical significance has been questioned.[14] Their results do not correlate well with the findings of Barker and others,[15] who viewed teeth with a polarizing

microscope prior to and following placing them for two five-minute periods into liquid nitrogen (-320F or -196C). Barker saw no changes in the irregularities which were present before testing, but did show that this extreme temperature change did create some new cracks.

Bachman and Lutz[13] also stated that using the scanning electron microscope (SEM), they were able to show changes in the surface caused by cold. However, this was not done systematically, but only as preliminary work to supplement the fluorescent dye findings. The SEM did appear to have potential for evaluation of the surface changes.

The purpose of this study was to evaluate the effect of the carbon dioxide snow on the tooth surface by utilizing both the scanning electron microscope and a surface analyzer. The surface analyzer (Surfanalyzer 1560, Federal Products, Providence, R.I.) is an instrument that passes a fine probe over the surface of objects and measures surface discrepancies at the micro-inch or micrometer level.

METHODS AND MATERIALS

Forty-five periodontally involved teeth were extracted without use of forceps to minimize additional traumatic forces being applied to the teeth. Fifteen of the teeth with the most apparent buccal craze lines were selected. The teeth were wiped clean and stored in normal saline. The buccal surface of each tooth was then polished for five seconds using fine wet pumice on a six inch buffing wheel at 3,000 RPM. A diamond scribe was used to scratch a small arrow on the gingival one-third of the buccal enamel surface for future orientation (Fig 1). An attempt was made to place the arrow so it pointed at or

was crossed by what appeared to be a fissure (Fig 2). Following arrow placement, the buccal surfaces were photographed at 6 and 12 magnification using a light microscope. With the light microscope, the surface scratches were usually indistinct while fissures were easily seen (Fig 3).

Several techniques have been successfully utilized to duplicate surfaces for SEM evaluation.[16-18] After evaluation of these techniques and variations of the techniques, the following technique was found to provide precise surface detail. In all cases, it easily picked up even the minute variations in the scratches made with the diamond scribe (Fig 2, 3).

Silicone impressions (Citricon, Kerr, Romulus, MI) were taken of the surface. Clear orthodontic resin was used to duplicate the surface using an incremental monoper-polymer build-up technique. The duplicate was then allowed to cure in hot water at 25 PSI.[19]

The tooth was then mounted in a fixed position relative to the surface analyzer probe. The probe could be run over the tooth surface at various speeds with the results being printed on a paper strip by a chart recorder. Readings were made so that the intervals present on the paper strips indicated 5 μm. The tooth was maintained in the same position relative to the probe during all testing. In each case, the probe was positioned to read the surface directly incisal to the arrow (Fig 3E,F,4-6). The CO_2 pencil (Odontotest, Union Broach), having a 3.5mm diameter, was then placed for 15 secs, 45 secs, and 1 minute intervals on the enamel surface at the tip of the arrow, and the surface was charted to each time interval. This gave a total of 2 minutes

of CO_2 cold on the surface. Since tooth position was not changed during testing, at each time interval the same area initially probed was reprobed (Fig 3-E). After testing, another silicone impression was made of each tooth and again the replica made using the cured, clear orthodontic resin. This gave pre- and post-testing models of the enamel surface. The models were mounted on aluminum stubs, coated with gold palladium (40-70 Å), and then evaluated by the scanning electron microscope (AMR 1000A Scanning Electron Microscope, Advanced Metals Research Corp, Wooten, Mass). Photographs of the surface were made at 30, 100, and 600 magnifications (Fig 2, 3B-D). Prior to and following testing, the teeth were stored in normal saline at room temperature.

RESULTS

In each of the fifteen cases with visual evidence of crazing, the fissures were clearly evident in the photographs taken with the light microscope. However, only nine had fissures actually present on the surface when evaluated by the SEM and surface analyzer (Table). In each case where the SEM showed a surface fissure, the surface analyzer also demonstrated the presence of a surface deviation 5 μm or more in depth in the buccal surface directly incisal to the inscribed arrow (Fig 4). The surface analyzer did not show surface deviations in the 5 μm range in any of the other six teeth where no surface fissure was shown by the SEM (Fig 5).

In each of the fifteen cases, the surface scratch was clearly

replicated in the duplication technique as shown by the SEM (Fig 2,3B-D). The surface analyzer also easily demonstrated the scratch (Fig 3F). The scratches made by the diamond had a charted appearance totally unlike the appearance of the fissures (Fig 3-F,4). The scratches were consistently broad (40 μm +) and shallow (5-20 μm), while the fissures were between 5-50 μm in width and depth, with increases in width usually associated with increases in depth.

After the two minutes of CO_2 snow testing, in no case could a new crack or surface deviation be detected by the surface analyzer or SEM on any of the 15 teeth. In no case, using the SEM, could a change in the crack's appearance be considered significant (Fig 2).

The surface analyzer detected an enlargement in only one of the nine cracks (Fig 6). It had an increase in both depth (< 20 μm) and width (< 10 μm).

In no case could the arrow scratched on the surface be shown to have changed in response to the carbon dioxide testing. All scratches were evaluated using the SEM and, in several cases, the tip of the arrow was analyzed using the surface analyzer probe (Fig 3-F).

After completion, several of the teeth were coated and evaluated directly by the SEM. In each case, the fissures shown by the replication and surface analyzer technique were present and matched. In no case could any real change from what was shown by the replication be seen (Fig 2).

DISCUSSION

All of the teeth in this study had the visual appearance of at

least one craze line in the enamel in the area of testing. Usually, several craze lines or fissures were observed. Yet, only nine of the fifteen teeth had measurable cracks in the area as demonstrated by the surface analyzer and, in each case, confirmed by the SEM. Other fissures were likely present, but only the surface at the tip of the arrow corresponding to the area of the cold testing was evaluated. Apparently, some of what appear to be surface fissures are actually deeper in the enamel, in the dentin, or just too small for the procedures employed to measure. The possibility of some craze lines being below the surface should be reasonable since the dentinal-enamel junction should be the area of highest stress concentration. This is true since it is an area of junction of two materials with different coefficients of expansion. Eisenstadt[20] has stated that areas of high stress concentration would be expected to have the highest number of craze lines or fractures.

With respect to the fissures actually present on the surface of teeth, two points need emphasis. First, since only teeth with an appearance of a high incidence of surface fissures were selected, the actual incidence (9 of 15) in the area of testing should be high relative to a random sampling of teeth. Therefore, during normal testing procedures, nowhere near this incidence of fissures should be encountered. Second, it must be recognized that, in this study, the surface analyzer probe and the electron microscope only evaluated the buccal surface adjacent to the inscribed arrow. This means that there could have been other surface craze lines. Still, the area evaluated by both techniques was the area where the testing was made so the major

changes occurring due to the testing should have been recorded.

Fissures too small to be picked up with the replication material would have to be very fine since even the many fine lines in the one to five micron range made by the diamond scribe were easily duplicated (Fig 2). Also, the surface analyzer easily demonstrated variations at the one to five micron level (Fig 3-4).

With respect to measuring the width and depth of the fissures, it is likely that the width of fissures were more exact than the depth. The depth of the fissures probably related to how deeply the probe could drop before reaching the other side, a limitation imposed by the size and geometry of the probe tip, or, possibly, by debris present in the fissures. Since the scratches were much shallower and broader, their measurement appeared very exact in both dimensions (Fig 3F).

It was interesting to note that, while the craze lines deep in the enamel or in the dentin were easily visualized in the light microscope photographs, the arrow scratched on the surface, easily seen by the scanning electron microscope on all the replicated surfaces, was often difficult to see using the light microscope photographs (Fig 3A, 7A-B). As shown by Figures 7C-D, this was not always true.

The only change shown by this study was an increase in depth ($<$ 20 μm) and width ($<$ 10 μm) in one of the nine fissures observed (Fig 6). Since this converts to about one-fiftieth of a millimeter in depth and one-hundredth of a millimeter in width, it appears Ehrman's question of clinical significance is reasonable.

Also, it should be recognized that the minute change in size of

the crack could easily be accounted for by just measuring a slightly different area of the crack. It is feasible that a slight movement could have occurred in the one case during the three episodes of testing. The probability that the change was caused by a slight movement of the tooth appears likely since no change was confirmed by the SEM.

The SEM photomicrographs at 100 magnification were excellent for evaluating the fissures (Fig 2B,E,H). At this magnification, very fine changes could be seen while exact orientation was still easy. At 600 magnification, correct orientation was more difficult (Fig 2C,F,I).

The finding of minimal changes does not appear to correlate well with the findings of Bachman and Lutz[13] and Lutz and others.[12] First, their studies showed a slight increase of cracks. In this study, no new cracks were seen. Second, they concluded that the existing cracks did change in width and depth. In this study, only one may have changed, and then only after a full two minutes of exposure to the cold. One explanation of the differences may relate to the fact that this study was *in vitro* while the others were *in vivo*. Perhaps the effect of carbon dioxide on teeth at body temperature, and hydrated internally by a blood supply, is greater. This does not appear likely since our continued *in vivo* work appears to support these findings of minimal effect.[21]

The only new cracks possibly shown in this study were in the actual teeth when, at the end of the experiment, some were evaluated directly by the SEM. While no change in the existing cracks could be seen, some

fine additional cracks did appear (Fig 2-G). It is feasible these cracks were not picked up by the replication technique, but it is not likely that both the replication technique and the surface analyzer would fail to show them. It is much more likely that the dessication of the tooth to allow viewing by the SEM created the new fissures. The finding of no change in existing fissures, but the creation of a few new fissures upon dessication appears to relate well with the findings of Barker and others.[13] They placed teeth in liquid nitrogen (-320 F) for two five-minute intervals and showed no change in existing cracks at the dentinal-enamel junction, but did create some new cracks. Also, if teeth are left in air for a few days, new fissures do occur.

Another explanation for the failure of this study to show changes in existing surface fissures could be explained if the cold only effects fissures deeper in the enamel or dentin. However, this would not explain the discrepancy from the previous studies, since they were describing changes shown by fluorescent dye also placed on the surface.

Another explanation could be that changes shown by the dye studies actually related to the spreading property of the dye. It is possible that each increase in cold caused a greater spread of the dye in existing, basically unchanged cracks.

Actually, the possibility that the carbon dioxide snow effects the enamel only minimally shouldn't appear too startling. In measuring the temperature of the snow, the coldest reading we could record was -56°C (-69°F), and some people spend a good deal of their lives at temperatures in this range.

It must be pointed out that while we tested the teeth for two minutes,

clinically, if no response is noted within ten to fifteen seconds, the tooth is generally listed as "no response."[13,14] In this study, the testing was continued for longer periods. Fifteen seconds was used to evaluate what might be the worst that could be expected clinically. After no changes could be shown after only fifteen seconds in preliminary work, the longer times were used to try and determine just when changes might be expected. Clinically, since most teeth respond in less than five seconds, most teeth receiving a test of ten seconds will probably prove pulpless and receive endodontic treatment. It is inconceivable that a tooth would be clinically tested for two minutes.

Many of the teeth, which do not respond within ten to fifteen seconds, will receive root canal therapy. This is then frequently followed by full coverage. From this viewpoint, the possibility of effect on the enamel in the 10-20 μm range seems truly insignificant. Furthermore, it took two minutes of testing to create even one possible change of this degree.

Somewhat interesting was the observation that the largest fissure located was leading into an amalgam incisal edge restoration (Fig 7A-B). Whether the amalgam restorations could have related to the original size of this fissure was not studied, but appears probable.

Finally, a comment on the difference in appearance of the scratches and the craze line appears indicated. The difference does conform to what Eirich[22] stated should be expected. The craze or fissure, which occurred naturally in the teeth due to internal stresses causing a sudden split, should have sharp, clean fracture lines.[22] Therefore, the probe

should tend to drop in and out in a smooth manner as was the case in this study (Fig 4). The scratches by the diamond scribe would tend to grind the enamel rods and prisms on the surface leaving a roughened surface.[22] This could give a broader, less clear cut area upon tracing with the surface analyzer. When the scratches were probed, this was clearly demonstrated (Fig 3F).

SUMMARY AND CONCLUSIONS

Fifteen teeth extracted atraumatically were selected for evidence of surface craze or fissure lines. Carbon dioxide snow was used to test each buccal surface for a total of two minutes of testing. The scanning electron microscope and a surface analyzer were used to test changes in the surface caused by the cold.

The findings were: CO_2 snow could not be shown to create new cracks or fissures on the tooth surface; the only change in the *in vitro* situation occurred after the full two minutes of exposure to cold. The change was a minute increase in size of one of nine surface fissures present in the areas of testing. This change was not confirmed by the SEM evaluations and was possibly due to experimental error.

In conclusion, it appears that the change, if any, caused by the CO_2 test as used clinically, is truly microscopic. This was true even after testing for a period of two minutes, which would be totally unacceptable clinically. When this is compared to the massive changes caused by simple operative procedures, it appears of minimal significance. The value of the diagnostic information created certainly appears of far greater significance. Therefore, with respect to dental enamel, carbon dioxide testing appears to be a safe procedure.

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Table. Correlation of apparent presence (+) or absence (-) of fissures by four evaluation techniques

Tooth #	Gross Exam.	Light Mic. Exam.	SEM	Surface Anal.
4	+	+	+	+
6	+	+	+	+
10	+	+	+	+
13	+	+	-	-
14	+	+	-	-
16	+	+	+	+
19	+	+	-	-
20	+	+	+	+
21	+	+	+	+*
22	+	+	-	-
25	+	+	+	+
28	+	+	+	+
35	+	+	-	-
37	+	+	-	-
41	+	+	+	+
Totals	15	15	9	9

*Only case where fissure appeared to enlarge after testing.

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LEGEND

Fig 1. Pictures of acrylic replica prior to (top) and following sputter coating (bottom) for evaluation by SEM. The arrow scratched on the surface is seen clearly following coating. Unlike this example, the arrows were generally scratched so they pointed towards the occlusal surface.

Fig 2. Scanning electron microscope examples of tooth #6 with fissure crossing the arrow scratched on the surface. Prior to testing: A. original magnification, X30; B. original magnification, X100; C. original magnification, X600. After testing: D. original magnification, X30; E. original magnification, X100; F. original magnification, X600. Actual tooth surface directly visualized by the SEM after testing and replication procedures: G. original magnification, X30 - note how fissure appears to become much finer occlusally just as shown in original replica (2A); H. original magnification, X100; I. original magnification, X600 - note that while at top, fissure might be considered wider than previous replicas (2C and F); the photograph is really of a more gingival location.

Fig 3. Example of tooth where visual examination and light microscope revealed multiple fissures, but not confirmed by SEM or surface analyzer. Tooth #35: A. light microscope showing multiple fissures (original magnification, X6); B. SEM (original magnification, X30); C. SEM (Original magnification, X100); D. SEM (original magnification, X600); E. surface analyzer

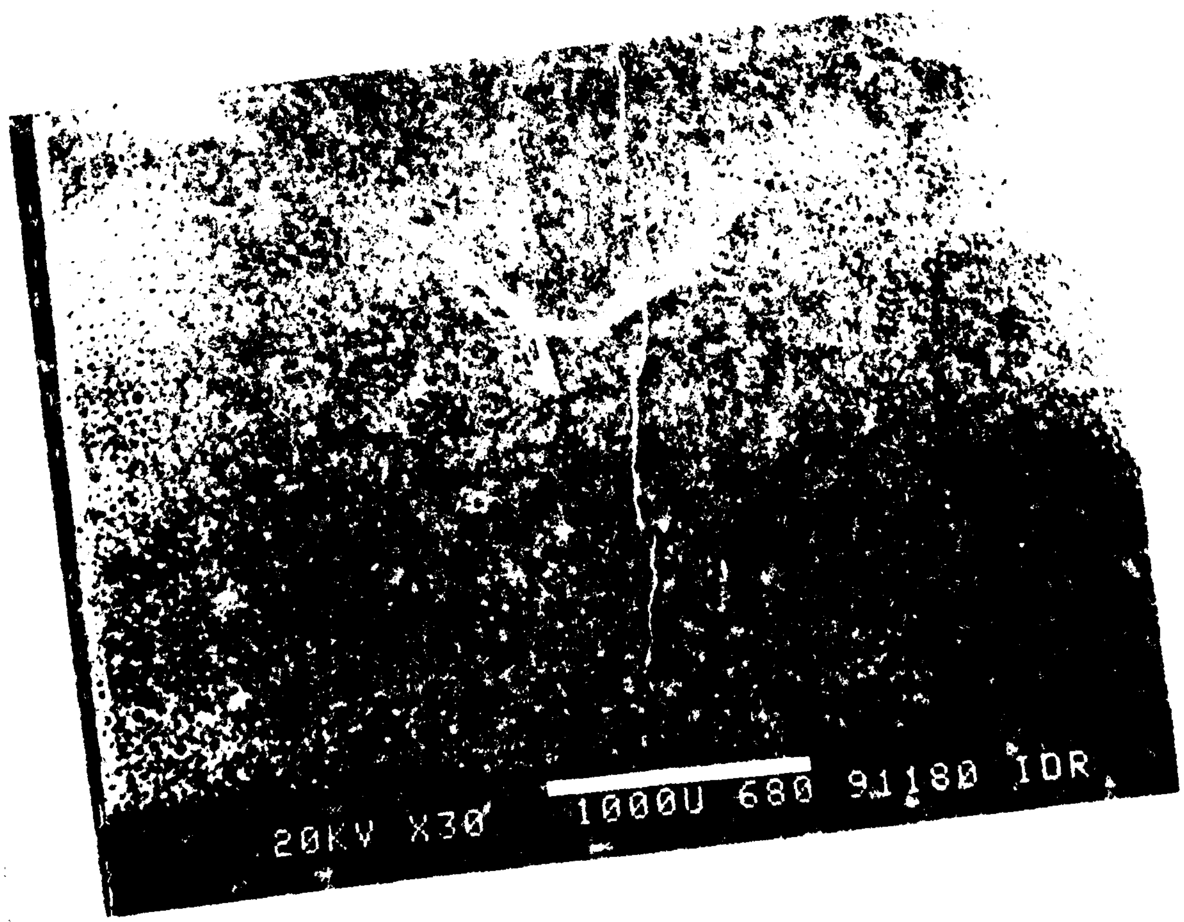
over surface before and after testing (intervals = 5 μm).
F. surface analyzer over tip of arrow.

Fig 4. Surface analyzer readings of four teeth prior to testing and after final test. Top left: largest surface crack in study. Bottom left: smallest surface crack in study. Right top and bottom: two medium size cracks in study (intervals = 5 μm).

Fig 5. Surface analyzer readings of two teeth which showed no surface deviations (intervals = 5 μm).

Fig 6. Surface analyzer reading of only tooth where fissure appeared to enlarge.

Fig 7. Light microscope photograph of buccal surface of tooth #28 which had largest fissure as read by surface analyzer and tooth #25 where arrow can be seen clearly and fissures appear extremely large. Tooth #28: A. original magnification, X6; B. original magnification, X12. Tooth #25: C. original magnification, X6; D. original magnification, X12.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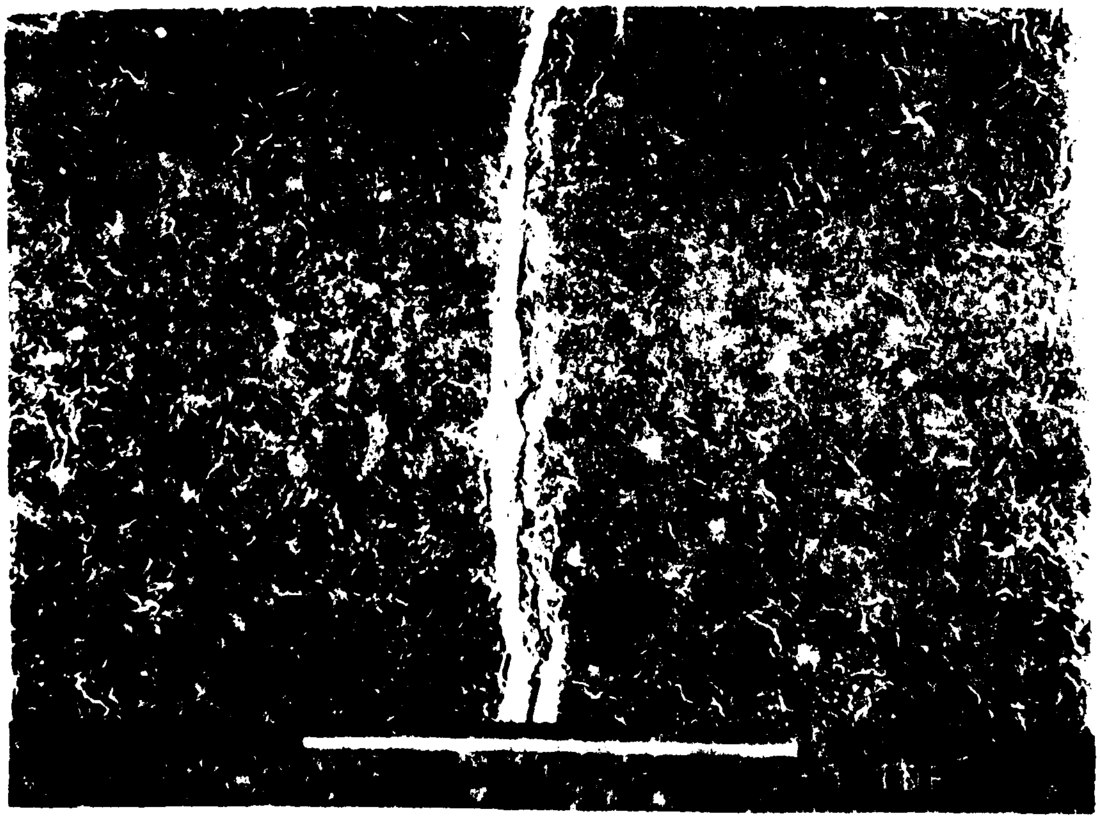

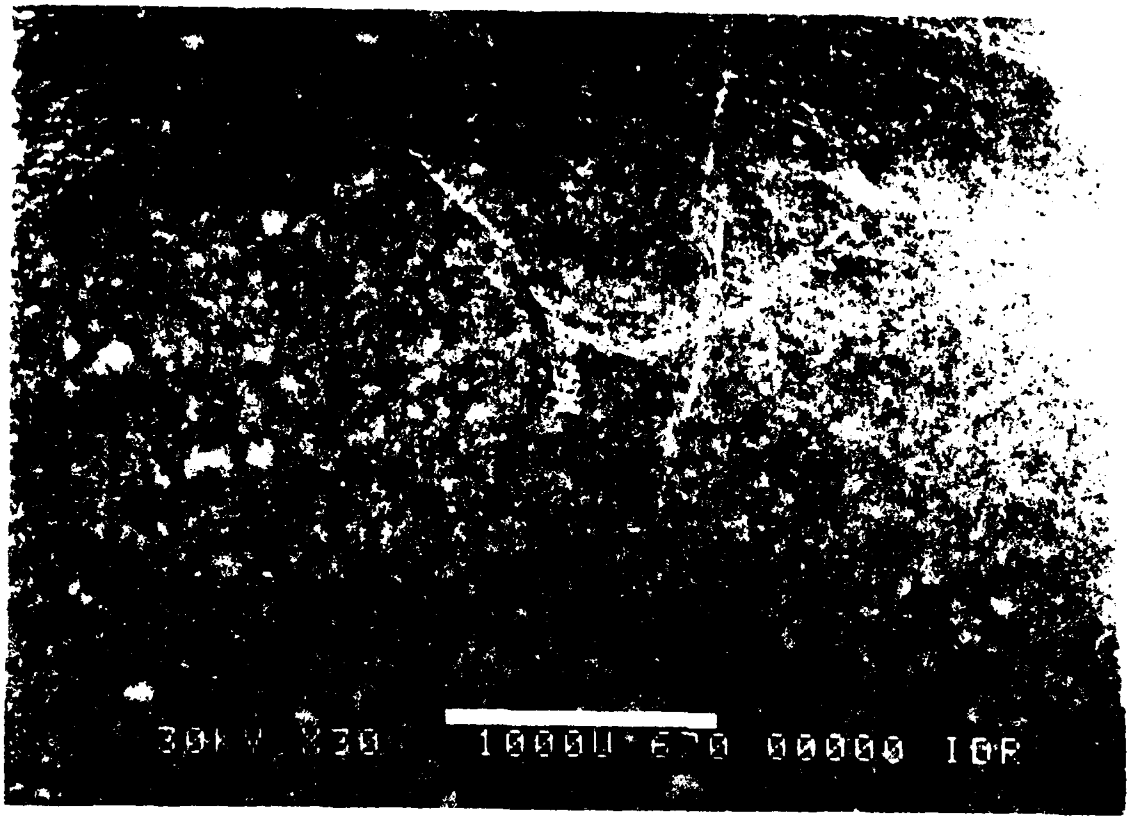
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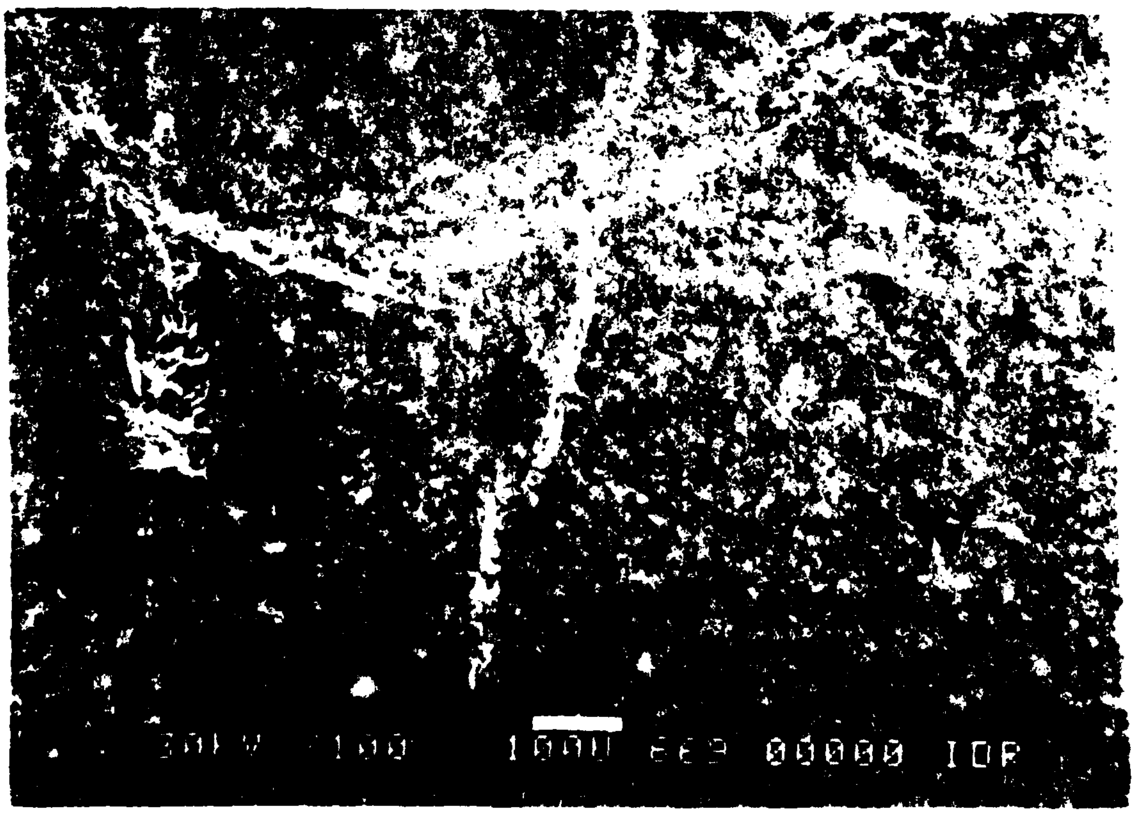
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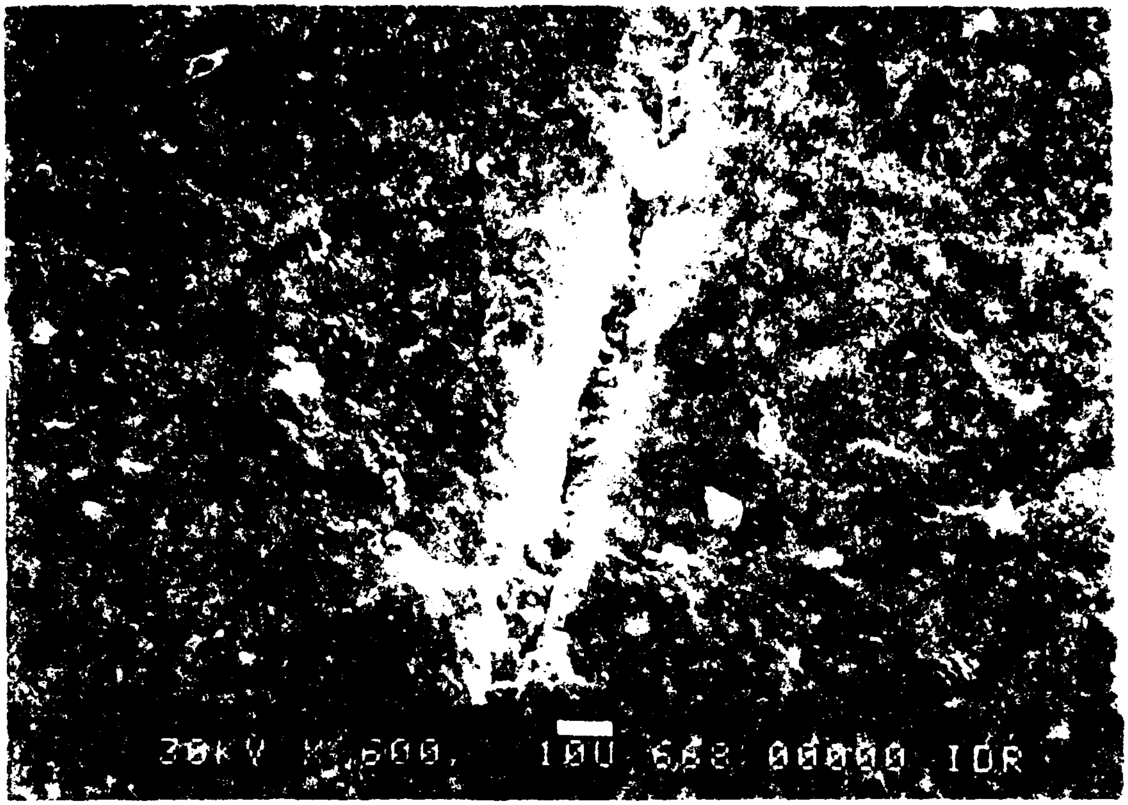
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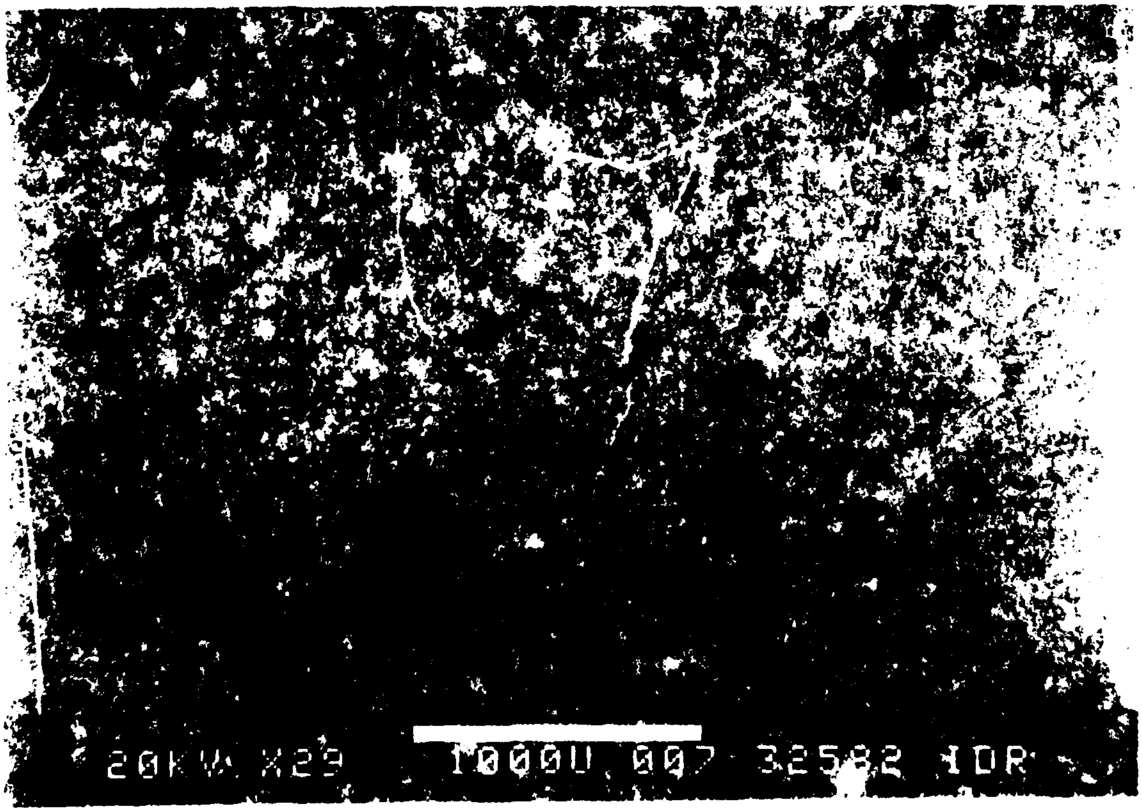
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Figure 3

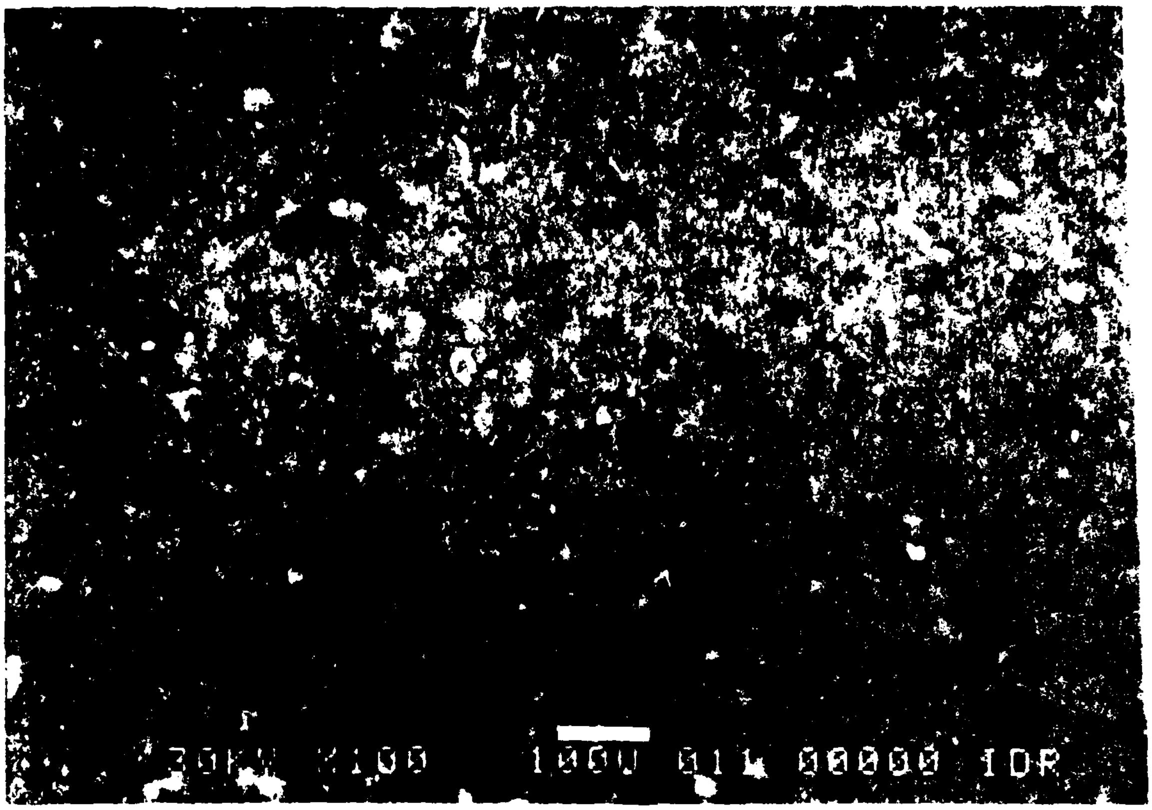

Figure [illegible]

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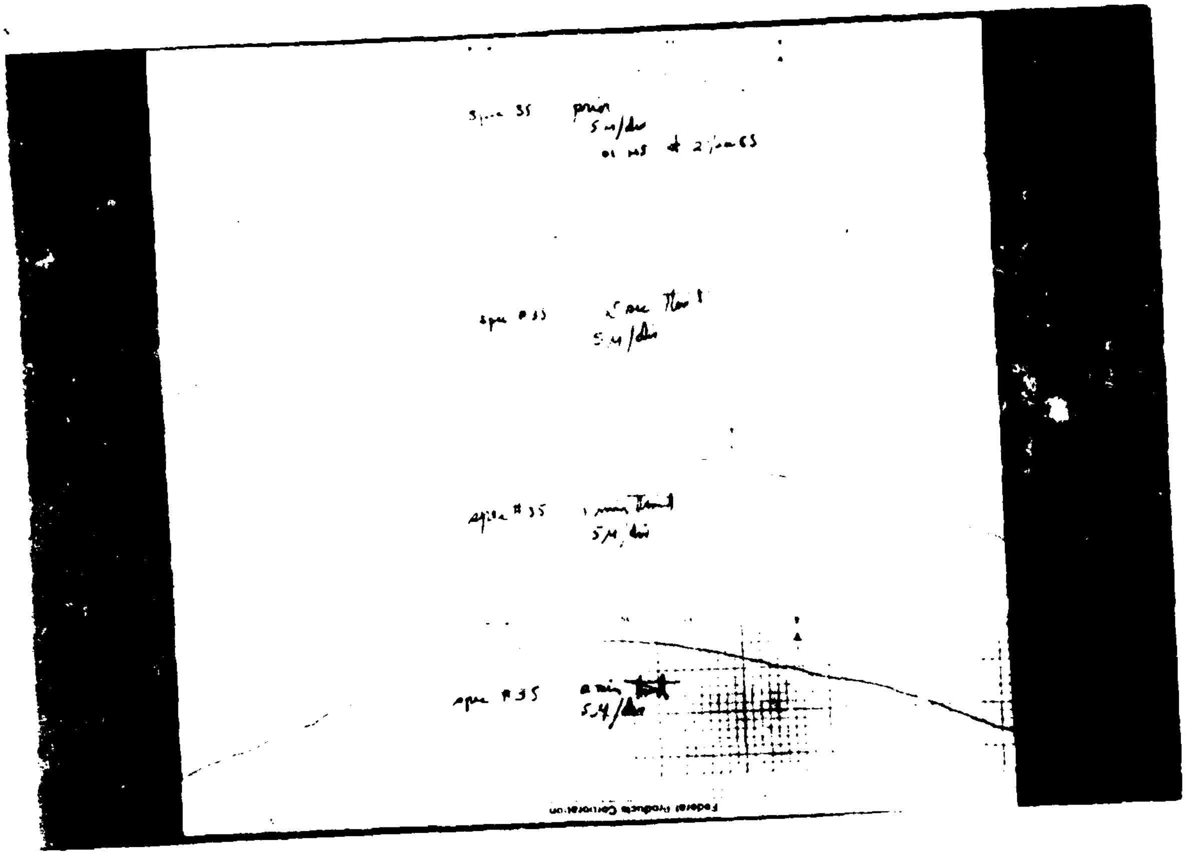

Figure 3E

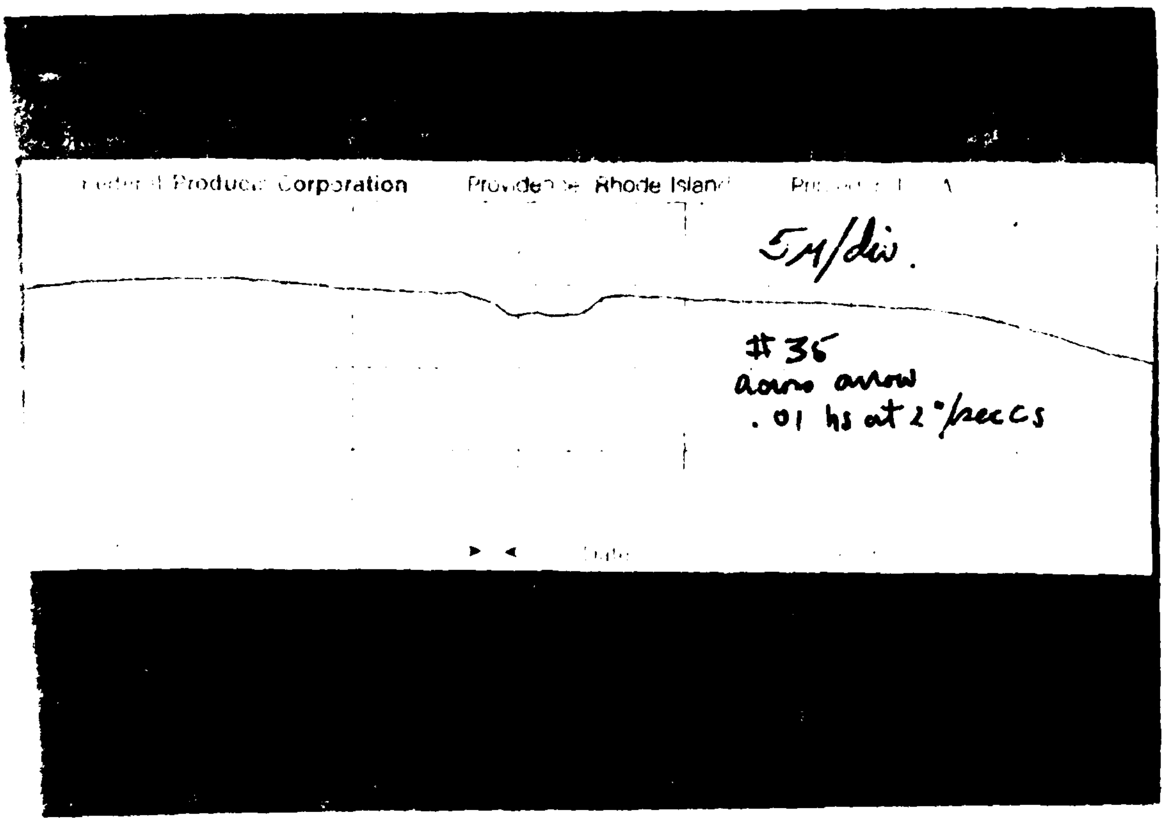

Figure 3F

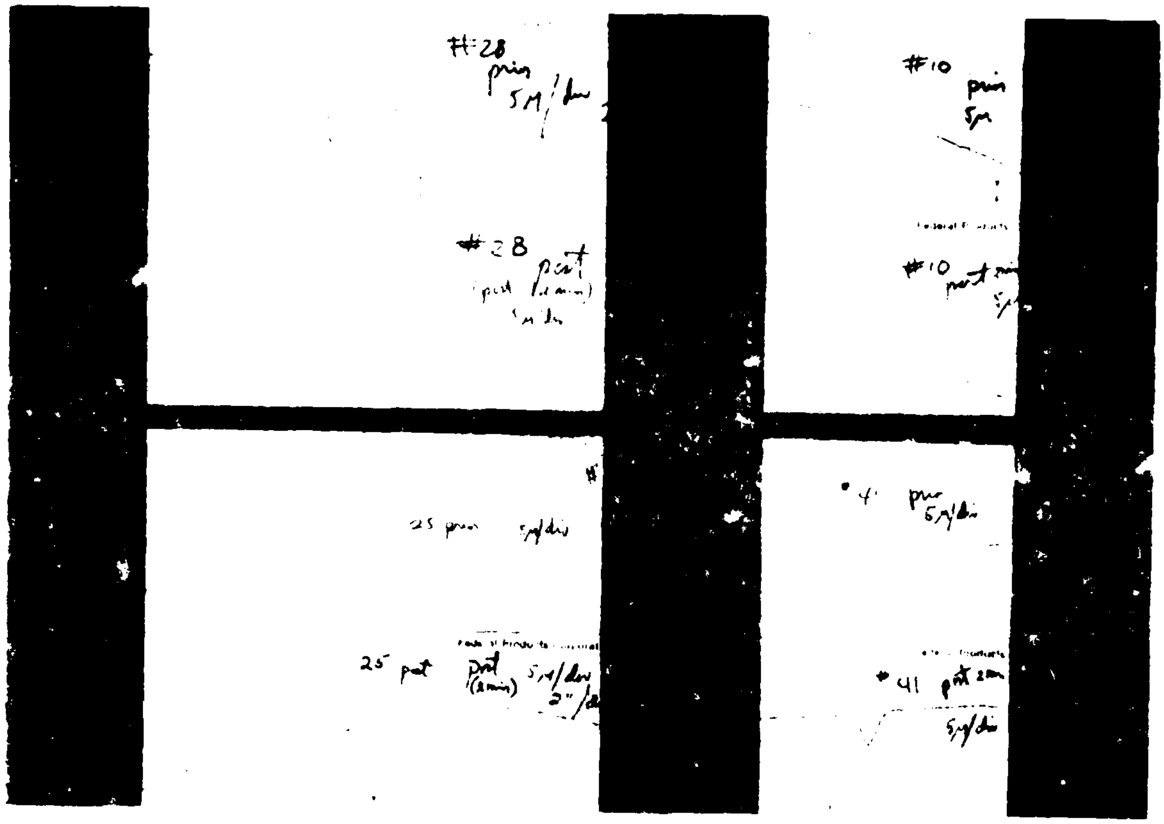

Figure 4

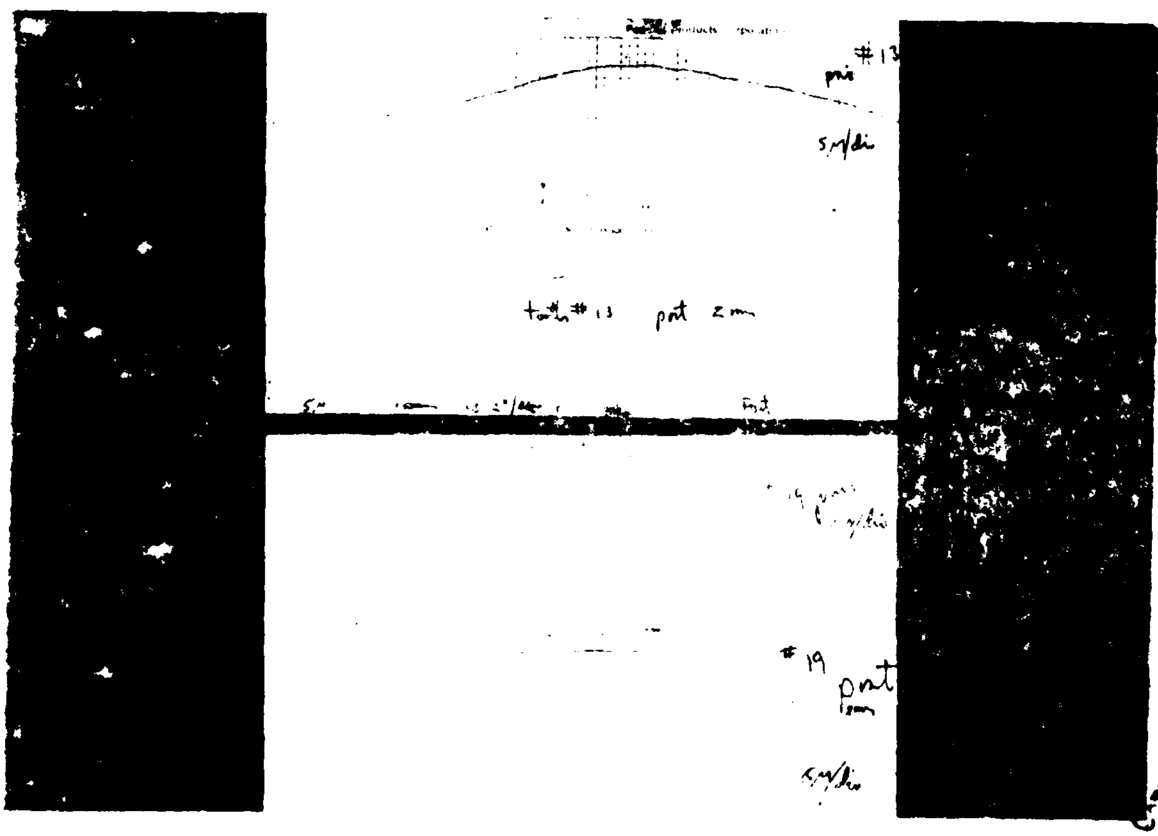

Figure 5

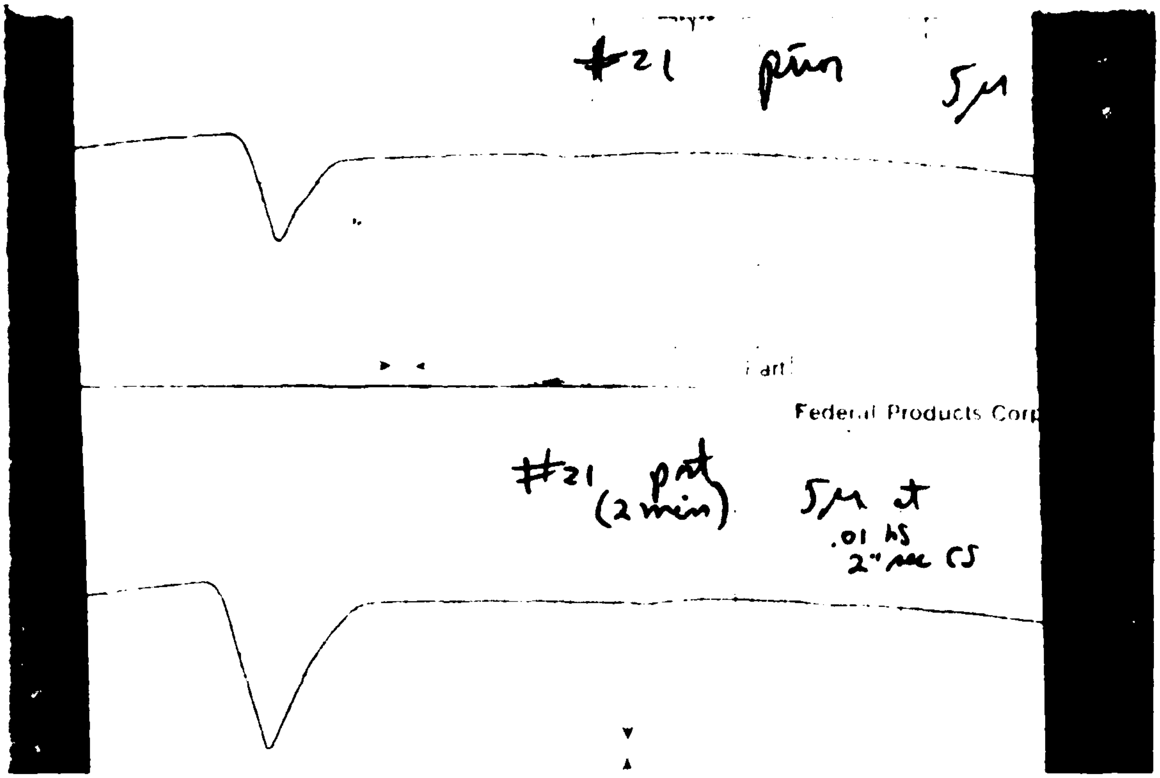

Figure 6

Figure 7A

Figure [illegible]

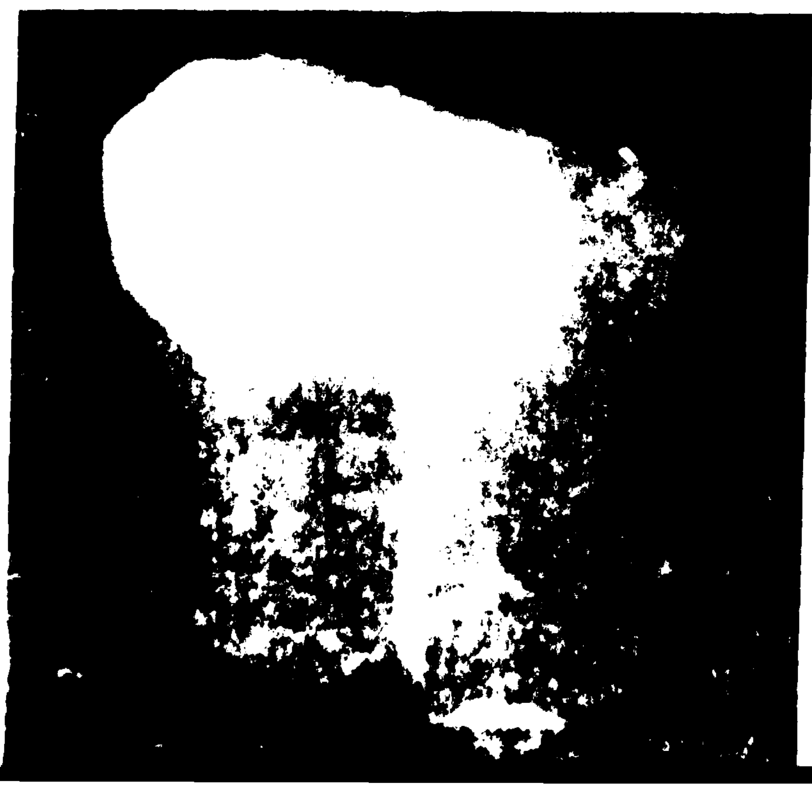

Figure [illegible]

Figure 7D

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